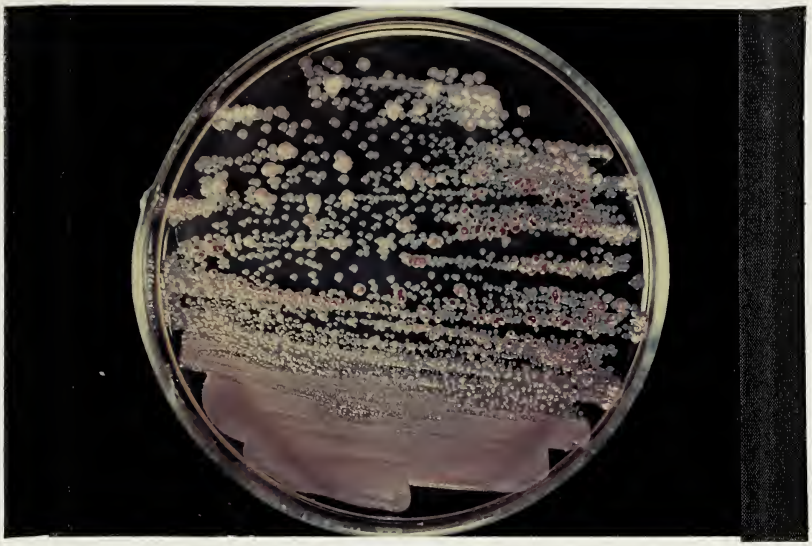
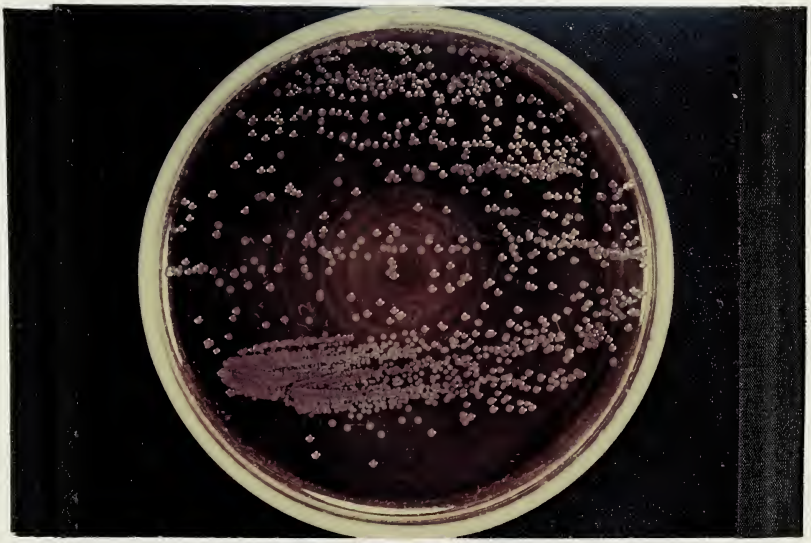
Margaret Christina Finlayson

THE VALIDITY AND IMPORTANCE OF THE GENUS PARACOLOBACTRUM AND ITS CONSTITUTIVE SPECIES



1. E. coli and P. aerogenoides



2. E. coli and Salmonella

Demonstrating the Similarity between P. aerogenoides and Salmonella when grown on MacConkey's agar.

THE UNIVERSITY OF ALBERTA

A STUDY OF THE VALIDITY AND IMPORTANCE OF THE GENUS PARACOLOBACTRUM AND ITS CONSTITUTIVE SPECIES

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

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by

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ABSTRACT

This investigation deals with the "Paracolon bacilli" as encountered in Enteric Bacteriology. An attempt has been made to determine the validity of the classification of such strains in the genus Paracolobactrum as suggested by Borman, Stuart and Wheeler (1944).

Cultural and biochemical as well as serological characters were investigated. Mention is made for comparative purposes, of the study of the pattern of protein metabolism as determined by "paper chromatographic" methods in a concurrent investigation by another worker.

The genus Paracolobactrum as defined by Borman, Stuart, and Wheeler was found to be valid. The validity of the species Paracolobactrum aerogenoides was also accepted. The species Paracolobactrum intermedium was, with one reservation, found to be valid. The validity of the third species, Paracolobactrum coliforme was questioned.

The similarity of the biochemical reactions of this genus and those of the genus Salmonella were indicated and the practical value of the adopted classification in differentiating between the two genera was demonstrated.

ACKNOWLEDGEMENTS

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I wish to express my appreciation to Dr. R.D. Stuart, Director of the Provincial Laboratory and Head of the Department of Bacteriology at the University of Alberta, for the opportunity to carry out this project and for his invaluable assistance and advice during the course of the investigation.

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PART ONE

INTRODUCTION

HISTORY

According to Sandiford (1935), the term "Paracolon" was first used by Gilbert and Lion in 1893 when they described a group of coliform bacilli found in the faeces of dysentery patients. These organisms did not ferment lactose, (time of incubation not stated) but they produced acid and gas in other test substances. They varied as regards indole production and motility.

Stuart et al (1943) tell us that the term was again used by Widal and Nobecourt in 1897 to describe a slow-lactose fermenting bacillus isolated from patients suffering from gastro-enteritis.

Since the beginning of the present century, reference to such organisms has become increasingly frequent in medical literature. Generally they have been described as paracolon bacilli but in certain instances as "aberrant coliforms". At the present time the term "Paracolon" seems to be preferred.

Articles by such authors as Seeliger (1951) working in Germany, Sevitt (1945) in Dublin, Sandiford (1935) in Cairo, and Stuart et al (1943) working in Providence, U.S.A. indicate that the occurrence of such strains is widespread indeed.

Unfortunately, however, with the increase in the number of references to these strains, the problem of their classification has at the same time become increasingly complicated. There seems to be not a little confusion between the various authors as to the definition of the paracolon bacilli.

The problem has become of sufficient importance to merit some discussion in recent text-books (Topley and Wilson - (1946) and Breed, Murray and Hitchens (1948)).

Topley and Wilson describe them as follows:

> "These organisms ferment lactose late, weakly, irregularly, or not at all. Some constantly give rise to non-lactose fermenting variants. Some produce gas abundantly, and some in only small quantity; others are completely anaerogenic. A few species are pathogenic for man; others are under suspicion; others again are almost certainly non-pathogenic. Some are found in faeces, some in water, some in soil and so some in other situations."

Breed, Murray and Hitchens describe the genus Paracolobactrum (Borman, Stuart and Wheeler (1944)) as:

> "Short rods characterized by consistently delayed fermentation of lactose (occasionally negative). Glucose is fermented with the formation of visible gas. Certain forms attack carbohydrates characteristically at 20°C to 30°C but not at 37°C. Antigenic relationships to other genera in the family are fairly common, even with respect to major antigens. The type species is Paracolobactrum aerogenoides."

CLASSIFICATION

In spite of the increasing importance of paracolon bacilli there has, as yet, been no international agreement on either definition or classification. In fact only a brief examination of the literature is necessary to indicate the variety of organisms to which the term "Paracolon" is applied.

Sandiford (1935) is one of several authors who regard paracolon bacilli as organisms giving acid and gas in glucose and mannite in 24 hours, no change in sucrose or lactose in the same time, and not agglutinating with "paratyphoid" antisera. The summary of Sandiford's article indicates that he considers indole production an additional feature necessary to the identification of paracolon bacilli.

Sevitt (1945) has studied a number of strains of paracolon bacilli with regard to their aetiological importance in an outbreak of infantile diarrhoea in Dublin. He defines paracolons as follows:

> "Gram-negative rods of the genus Bacterium which either fail to ferment lactose, or ferment it late or irregularly. They attack other sugars however, producing acid or acid and gas. Biochemically they usually differ from the Salmonella and dysentery groups. They differ from Proteus in failing to spread on solid media if motile. Most strains fail to liquefy gelatin and do not produce H_2S. Most paracolon bacilli closely resemble the coli-aerogenes group, but differ from members of the group in their failure to or slowness to attack lactose."

He divides his strains into four groups as shown in Table 1.

Table 1

Classification of Paracolon bacilli - Sevitt (1945)

Group	No.strains	Glu.	Mal.	Man.	Dul.	Suc.	Ind.	Mot.
1	50	AG	AG	A(AG)	+	-	V+	nil
11	14	AG	AG	AG	-	-	+	mostly nil
111	28	AG	AG	AG	+	AG	V+	mostly nil
sub 111	5	AG	-	AG	+	AG	V	nil
1V	11	AG	AG	AG	-	AG		mostly nil

Glu. = Glucose
Mal. = Maltose
Man. = Mannite
Dul. = Dulcite
Suc. = Sucrose

Ind. = Indole
Mot. = Motility
\+ = Acid and gas, later becoming alkaline
V = Variable
V+ = More often positive

Schwabacher (1949) in a biochemical classification makes a preliminary division on the fermentation of sucrose. Her classification can be represented by Figure 1.

After using such a tentative classification she states that:

> "There was no constant association between biochemical and serological groups. Nor was there an antigen common to all or even the majority of paracolon strains."

In neither of these classifications are "generic" or specific definitions given. The biochemical reactions listed are somewhat limited in each case as they do not include such reactions as the

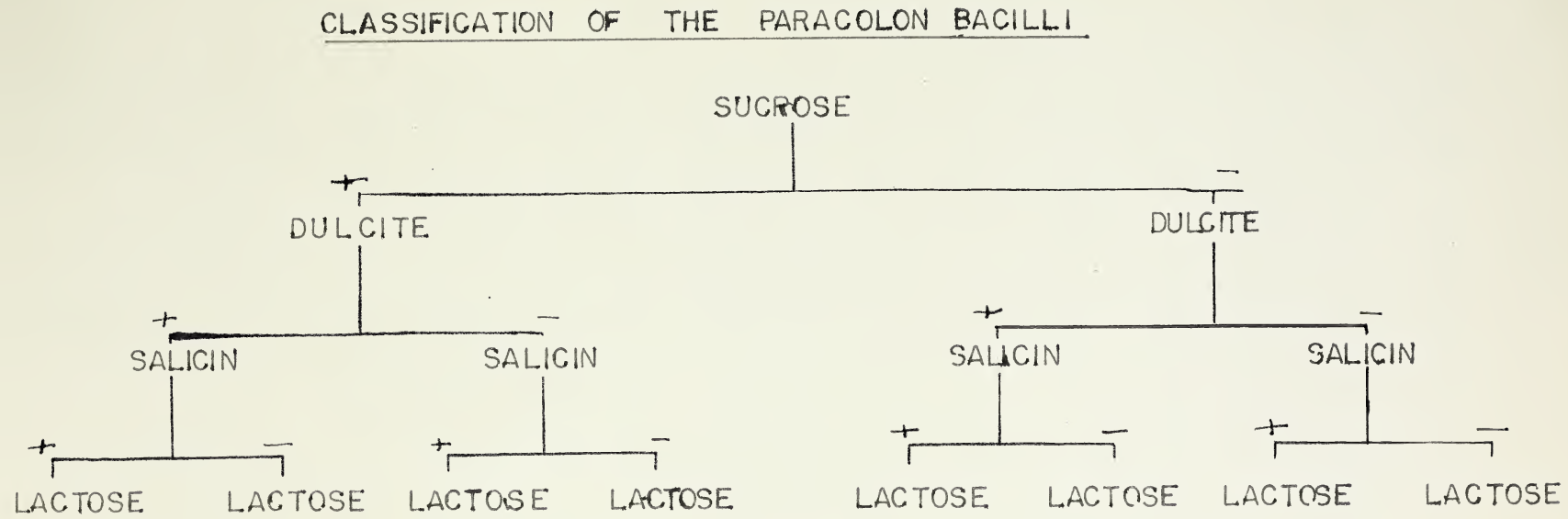


Figure 1

Classification of the Paracolon Bacilli

As suggested by Schwabacher (1949)

"Imvic" group. The strains studied are varied and are occasionally quite dissimilar.

Borman, Stuart and Wheeler (1944) have suggested a considerably more extensive classification than any of these just considered. Not only is it more extensive but genera and species are well defined. In their article entitled "The Taxonomy of the Enterobacteriaceae" they have to a certain extent rearranged the entire tribe of Enterobacteriaceae and they have added several new genera. One of these is "Paracolobactrum" which seems to cover the paracolon bacilli as they are generally identified.

Figure 2 represents their suggested classification.

The genus Colobactrum is defined as follows:

> "Aerobic, non-sporing gram-negative short rods. Fermentation of glucose and lactose with formation of visible gas in 24 hours at 37°C. Widely distributed in nature as saprophytes. A common antigenic pattern is not discernable within any one biochemical type."

The genus Paracolobactrum is defined as follows:

> "Aerobic, non-sporogenic, gram-negative rods characterized by consistently delayed fermentation of lactose (may occasionally be negative). Glucose is fermented with the formation of visible gas. Certain formsattack carbohydrates at 25°C but not at 37°C. Antigenic relationships to other genera in the family is fairly common, even with respect to the major antigens."

In each case the definition is ammended by subdivisions as in Table 2.

TAXONOMY OF THE ENTEROBACTERIACEAE

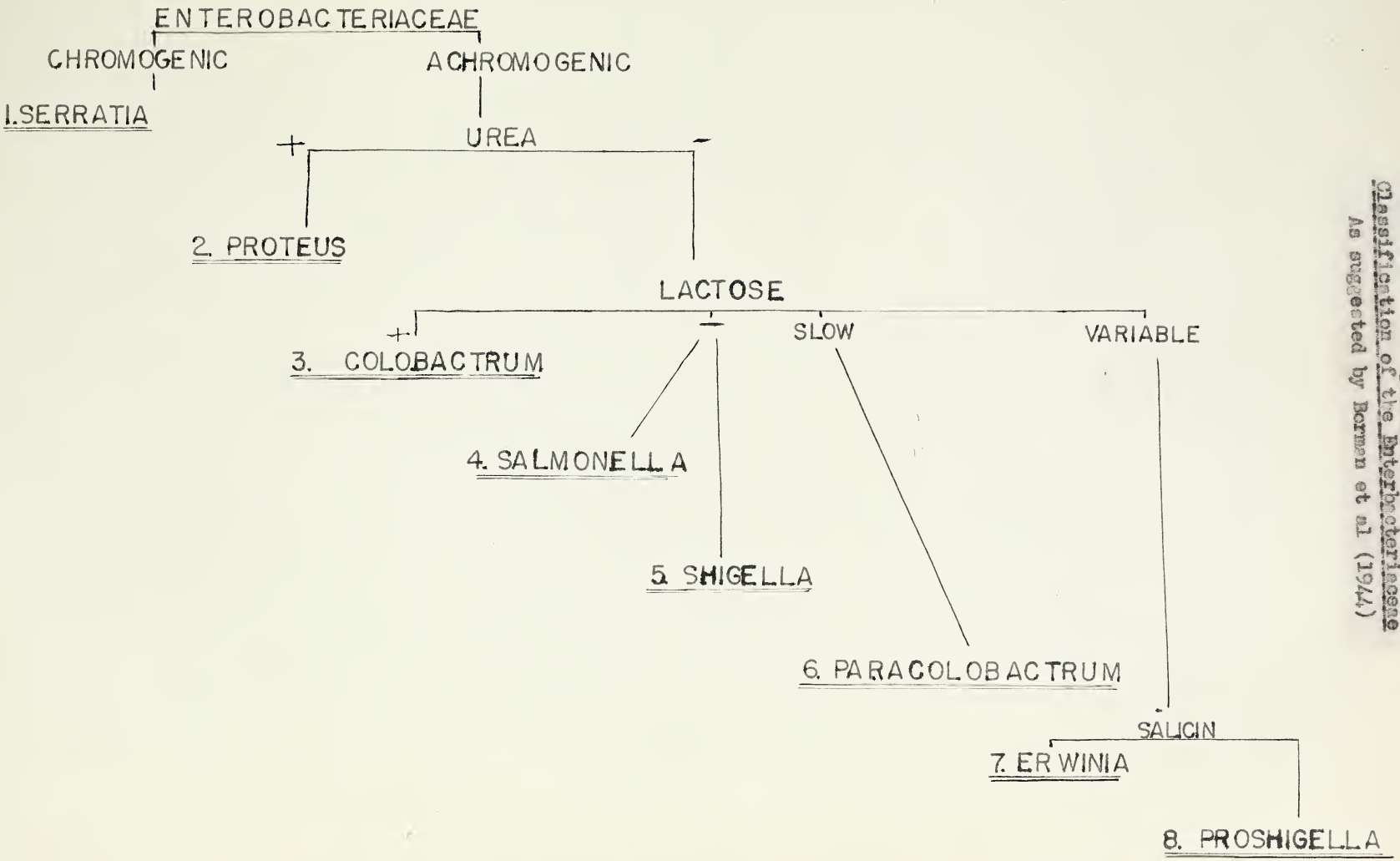


Figure 2
Classification of the Enterobacteriaceae
As suggested by Borman et al (1944)

Table 2

Divisions of the Colobactrum and Paracolobactrum genera

	Colobactrum	Paracolobactrum
Acetylmethylcarbinol	aerogenes	aerogenoides
Acetylmethylcarbinol not produced A. citric acid utilized	freundii	intermedium
B. citric acid not utilized	coli	coliforme

In this investigation the classifications of Borman et al will be followed with a view to determining its adaptability and practicability for use in a routine Public Health Laboratory.

The intermedium group has already been investigated serologically by several workers, Bruner, Edwards and Hopson (1949), Edwards, West and Bruner (1948), West, Edwards and Bruner (1947a) and(1947b). Accordingly an attempt has been made to determine the serological relationships between members of the P. aerogenoides group as found within this Province.

PART TWO

METHODS

METHODS

1. Collection of strains

The majority of the cultures investigated were derived from faecal and urinary specimens received in the Enterobacteriology Department.

Specimens were planted directly to MacConkey and SS agar plates (Difco) and were introduced into tetrathionate broth. To the latter about 1 gram of faeces was added or up to 3 cc of urine and the plates together with the tetrathionate broth cultures were incubated overnight at 37°C. On the following day, subcultures were made from the tetrathionate broth to MacConkey and SS plates. In all cases the plates were examined after overnight incubation for the presence of white (non-lactose-fermenting) colonies. If such colonies were present they were picked to lactose broth and peptone water. Two picks were made from each plate unless the colonial morphology suggested that there were more than two types of colonies present - in this case a suitable number were picked to include all types present on the plate. Colonies of dubious non-lactose fermenting qualities were also picked to exclude the possibility of overlooking possible pathogens.

Further biochemical reactions were determined on the strains which were negative in lactose broth in 24 hours. These reactions included fermentation of glucose, sucrose, mannitol and dulcitol as well as the production of hydrogen sulphide and indole. The original lactose broth was read again after 48 hours incubation.

Proteus, Pseudomonas and Alkaligenes were recognized on

these reactions. Salmonella and Shigella were suspected on these reactions and confirmed by appropriate serological tests. There remained a group which fell into one of the following three categories:

1. Organisms which fermented both glucose and mannitol with the production of acid and gas, and which, because of a positive indole reaction, were excluded from the Salmonella group.

2. Organisms which fermented both glucose and mannitol with the production of acid and gas and which, because of a negative agglutination test were excluded from the Salmonella genus.

3. Organisms which fermented glucose only with the production of acid and gas.

This group formed the "probable Paracolons" and further biochemical reactions were determined as follows:

1. Fermentation of arabinose, xylose, sorbitol, inositol, rhamnose and salicin.

2. Utilization of citrate and urea.

3. Fermentation of sodium d-tartrate.

4. Gelatin liquefaction.

5. Reaction in litmus milk.

6. Determination of Voges Proskauer and Methyl Red reactions.

These tests permitted a final identification of "Paracolobactrum" strains and the others were discarded from this investigation. In this work the "Paracolobactrum" do not include any sucrose positive strains nor any anaerogenic strains. It was found early in the investigation that sucrose positive strains occurred

very frequently and to include them would have given too many cultures to handle conveniently. By the definition given earlier, Paracolobactrum is aerogenic. The majority of anaerogenic strains can probably be classified under "Proshigella" and do not concern this investigation.

2. Maintenance of cultures for study.

In many instances a considerable time interval was likely between the isolation and subsequent studies of the organisms, so it was necessary to adopt a method of maintaining them in culture until required. All strains to be kept were planted on egg saline slants, incubated overnight, sealed with paraffin wax, and stored in the refrigerator. Numbers of transplants varied. In some cases none were made.

46 of the cultures isolated in the early part of the investigation were dried and maintained in that condition until required. The method of Stamp and Stone (1947) was adopted. It is not only simple but requires relatively little time.

A large number of these dried cultures were transplanted after a time interval of approximately two years. The discs were placed in plain broth and incubated at 37°C. overnight. In all but three instances good growth was obtained. In these three instances new discs were placed in serum broth and incubated as before. Good growth was then obtained with these also.

3. Serological methods.

A. preparation of antisera.

Both "H" and "O" antisera were prepared in this investiga-

tion and in both cases the method of Edwards and Bruner (1942) was used.

The antigen used for preparation of an "O" antiserum was a 24 hour broth culture boiled for two hours and preserved by the addition of 0.4% formalin. It was found that the use of standard amounts of broth (30cc) gave antigens of comparable and suitable density and standardizations for density was not carried out.

Fully grown male rabbits were used, and injections were given intravenously using the marginal vein of the ear. The primary dose was 0.5 cc and injections were given at five day intervals doubling the dosage each time to a maximum of 5 cc. A trial bleeding was done five days after the fifth injection and a good titre was obtained in most cases. A few animals required a sixth injection but in only one case was a good titre not obtained at this time. When a satisfactory titre was obtained, the animal was bled and the serum preserved with merthiolate. The majority of animals stood the injections well. In the latter part of the investigation a few animals experienced severe reactions following the initial dose. The initial dose was then reduced to 0.25 cc doubling the dose for further injections as before.

"H" antisera were prepared in somewhat the same manner. Antigens were again 24 hour broth cultures, this time preserved immediately by the addition of 0.4% formalin, without boiling. Strains to be used for "H" antigens were passed through soft

agar several times to ensure the development of forms with maximum flagellar substance for use as antigen. The primary injection was 0.25 cc. A good titre was obtained after three injections rather than five. The animals were bled and the serum again preserved by the addition of merthiolate.

4. Agglutination tests.

a) "O" agglutination tests.

Two methods were commonly employed in the performance of "O" agglutination tests. The tube method employed boiled broth antigens similiar to those used for the preparation of antiserum. Tubes used were 10 x 75 mm and 1 cc each of antiserum dilution and antigen was placed in each tube. These were incubated for 4 hours at 52°C and then overnight at room temperature. Tests were read with the aid of a fluorescent light againsta dark background and were considered positive when the supernatant fluid had become clear and the organisms had formed a granular sediment at the bottom of the tube. Antiserum dilutions varied with the particular test being carried out.

The slide method employed in almost all cases a 1/10 dilution of the antiserum in saline and the antigen was supplied by the suspension resulting from the suspension of the growth from an agar slant (15 x 120 mm tubes) in not more than 1 cc of normal saline. To a drop of antiserum in a hollow ground slide a drop of the antigen suspension was added and the mixture was mixed first with a wooden applicator and then rotated for several seconds. The reaction was observed with the aid of fluorescent light against a

dark background over a period of not more than two minutes. Agglutinations were recorded as ++++ (complete) to + (doubtful).

b) "H" agglutination tests.

These were always carried out as tube tests. The antigen was prepared in the same way as that used in the preparation of antisera. 1 cc each of antigen and antiserum dilution indicated were placed in a 10 x 75 mm test tube in a 52°C water bath and incubated for one hour. At the end of this time the tests were read with a fluorescent light against a dark background. A positive test showed clear fluid with loose, flucculent clumps of organisms. These tests were not allowed to stand for more than fifteen minutes so that cross reactions due to "O" agglutination did not become evident.

5. Absorption tests.

Preliminary tests with "O" antisera of high titre and their homologous antigens indicated that the following method supplied sufficient absorbing suspension to absorb all antibodies present.

For each absorption four antigen bottles were used. These were twelve ounce flat bottles with a slant of plain agar on one side. They were inoculated with a suspension of the organism in broth using about 1 cc of suspension for each bottle. The surface of the agar was covered with the fluid to ensure uniform inoculation and then the bottles were incubated for 24 hours in such a position that the fluid remained on the surface of the agar. On the following day 10 cc of normal saline was added to each bottle and the growth suspended in the fluid. The suspensions were collected in tubes and centrifuged. The clear supern-

atant was discarded and the sediment used as the absorbing suspension. To half of the suspension 1 cc of serum to be absorbed was added. This was placed in a 52°C water bath for two hours. After centrifuging the supernatant was removed and added to the other half of the absorbing mixture. The process was repeated and the supernatant from the second incubation was the absorbed serum. This serum was cleared with chloroform and stored in the refrigerator for testing. The usual type of "O" tube agglutination was used to determine the efficiency of the absorption in each case.

Procedure for "H" serum was almost identical except of course that the procedure for "H" agglutination was used in determing the efficiency of the absorption.

PART THREE

A SYSTEMATIC STUDY OF THE CULTURAL AND METABOLIC CHARACTERS OF THE GENUS *PARACOLOBACTRUM*

INCIDENCE OF PARACOLON BACILLI AND FACTORS RELATED THERETO

1. Incidence of Paracolon bacilli

The discussion of the classification of the paracolon bacilli indicated the variations in the definition of this term when discussed by different authors. It is therefore difficult to draw any comparisons regarding the incidence of such organisms from figures quoted in different papers.

Sandiford (1935) working in Cairo, found that of faecal specimens sent to his laboratory for culture for various reasons, 16.1% of those examined for dysentery, 10.4% of those for enteric fevers, 8.6% of those considered normal and 4.4% of urines yielded paracolon bacilli. He did not include sucrose fermenting or indole negative strains in this investigation.

Sevitt (1945) working in Dublin on the problem of infantile diarrhoea, found that 26.2% of the faecal specimens from patients and 11% of the controls (normal) yielded paracolon bacilli. He apparently did include sucrose positive and both indole positive and indole negative strains. It must be pointed out that in his work he was dealing with a very limited age group, up to two years of age only.

In Edmonton, during the year 1950, 794 "enteric" specimens were received. On culture, 76 or 9% yielded Salmonellae or Shigellae and 239 or 30% yielded strains that fell into the "probable paracolon" group. Of these, 91 or 11% were actual Paracolobactrum strains (according to the definition given on page 5).

2. Incidence of Paracolobactrum species

To the above paracolons were added 77 cultures isolated by the general bacteriology section of the Laboratory and 16 cultures isolated by the Provincial Veterinary Laboratory. These strains were isolated during the same period and were included for purposes of comparison.

The strains from the general bacteriology section (identified by the letter M) were from a variety of sources such as urines, sputums, wound swabs and foodstuffs.

Cultures received from the Veterinary Laboratory(identified by the letters AP) were from either animal or avian(poultry) sources. They were isolated during the course of routine examinations carried out by that laboratory.

A total of 334 strains were examined and classified as "probable paracolon." Of these, 119 were found to be Paracolobactrum strains and the others were discarded for a variety of reasons. Some discarded strains were anaerogenic and some mannite negative. Many were urea positive or did not correspond to the definition in other respects.

Table 4 gives the source of strains and their specific identity in the genus Paracolobactrum.

Table 4

Source and Specific Identification of Paracolobactrum strains.

Identity	"Enteric"	"General"	"Veterinary"
Paracolobactrum coliforme	46	8	12
Paracolobactrum aerogenoides	35	4	2
Paracolobactrum intermedium	10	0	1
Totals	91	12	16

The work of Stuart et al (1943) offers some comparison on the incidence of the various strains.

They examined all paracolon strains submitted to them at Brown University in Rhode Island, U.S.A., over a period of five years. Of 465 strains considered, 77.4% were from patients suffering from gastro-enteritis, 18.1% from food handlers, and 4.5% from "others."

Table 5 compares the identity of the strains encountered.

Table 5

Comparative incidence of Paracolobactrum strains.

Strain	Brown University	Alberta
P. coliforme	223 or 55%	48 or 48%
P. aerogenoides	140 or 35%	35 or 35%
P. intermedium	40 or 10%	15 or 16%
Totals	403	98

The percentage of P. aerogenoides is the same in each case, although the comparison is not entirely justifiable, since the number of strains considered in the Brown University survey is approximately four times greater than in this investigation. It might also be pointed out that almost half of the P. intermedium strains met with in Alberta, originated in the same institution.

The workers in the Brown University survey said that they saw some evidence of geographical segregation within the paracolon group since "with few exceptions, strains from one locality were frequently identical with cultures from the same, but seldom with the strains from different localities".

3. Effect of Differential media on Incidence

Some authors have suggested that the incidence of paracolon bacilli has increased with the introduction of various "enrichment media". Table 6 indicates the incidence of the various species in relation to the media used in this investigation.

Table 6

Comparison of efficiency of various media in isolation of paracolons

Media	P. int.	P. aer.	P. col.	Total
Direct only (MacConkey and SS)	6	17	34	57
Tetrathionate only (MacConkey and SS)	6	8	7	21
Direct and Tetrathionate	4	10	6	20
	16	35	47	98

P. int. - intermedium P. aer - aerogenoides P. coli - coliforme

Table 6 shows that P. coliforme was isolated most frequently from the direct plates, and especially from direct MacConkey. The isolation of P. aerogenoides was not so significantly related to the type of culture medium used, but there was certainly no evidence that it was particularly favoured by the "enrichment medium".

OBSERVATIONS ON THE CULTURAL AND BIOCHEMICAL REACTIONS OF THE GEUNS PARACOLOBACTRUM

During the time interval that elapsed before special studies were undertaken, a few strains died. As a general rule, only one strain from each patient was kept for special investigation. For these reasons the number of strains remaining available for special investigation is less than the number indicated in Table 4.

All strains were gram negative bacilli of the usual "coliform" type. Morphologically, there was some difference between species. P. coliforme strains were somewhat shorter and fatter than the other two species; P. aerogenoides occasionally showed rather uneven staining which could be described as "barring".

On MacConkey's and SS agar the colonies frequently had a slightly brownish tinge, but in no instance could they be regarded as lactose fermenters.

Table 7 represents the biochemical reactions encountered in the strains reserved for special study. It also indicates their identity according to the Borman scheme. Several points stand out. The absence of sorbite and inosite fermentation was a general character applicable to the genus. Hydrogen sulphide production on the other hand was limited to the intermedium group, as was with one or two exceptions the capacity to ferment dulcite.

The variations between the general biochemical characters do not vary more than those of established species within the genera Salmonella and Shigella.

Occasional strains possessed fermentative activity considerably different from their respective species patterns. The problem

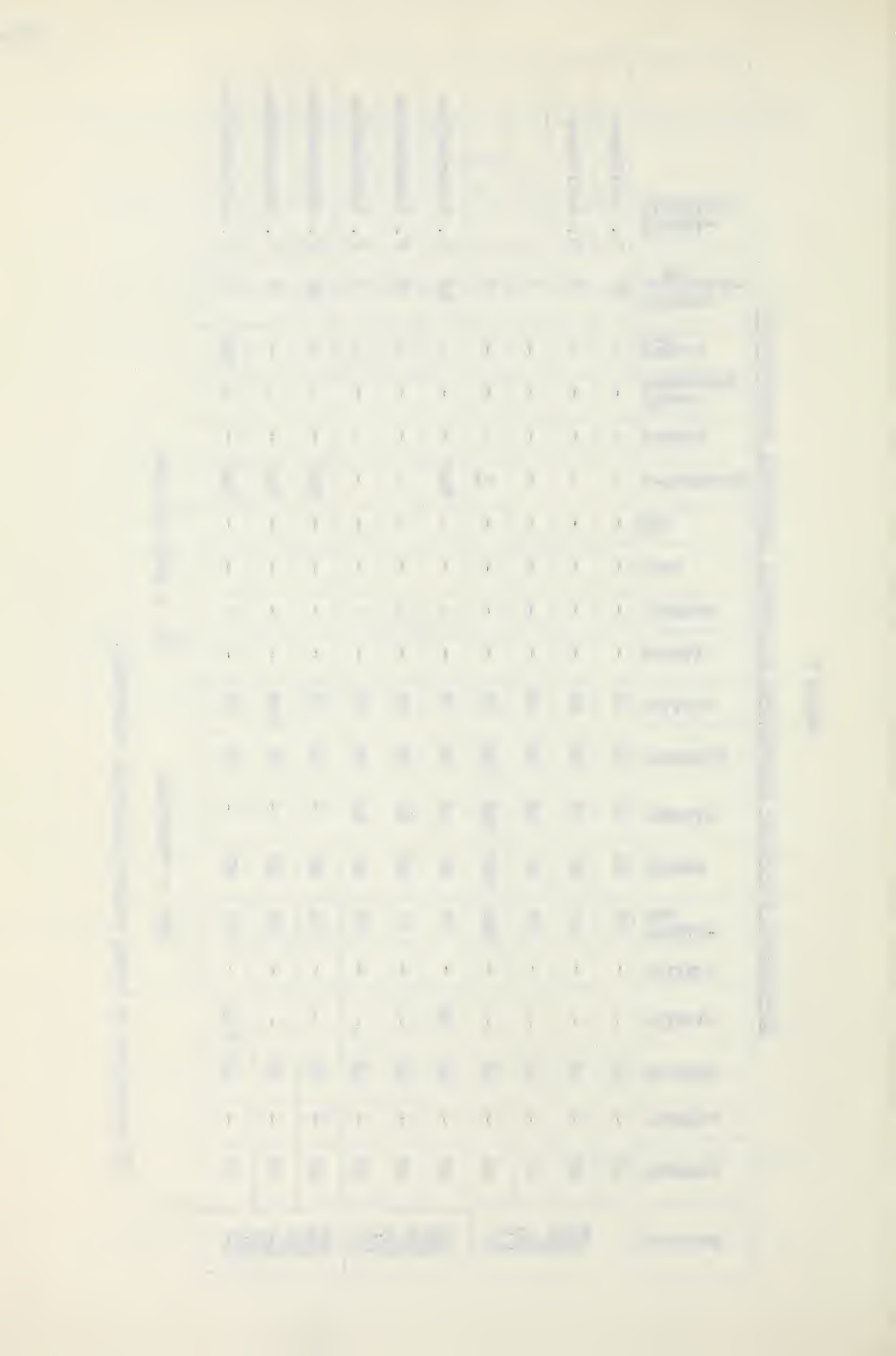
Table 7

Biochemical Reactions encountered in strains specially investigated

Lactose	Glucose	Sucrose	Mannite	Dulcite	Salicin	Arabinose	Xylose	Sorbite	Rhamnose	Maltose	Inosite	Citrate	Urea	H_2S	d-Tartrate	Indole	Voges Proskauer	Methyl Red	Numbers encountered	Identification
Ag-average 2-10 days	AG	-	AG	-	-	AG	AG	AG	AG	AG	-	-	-	-	+	+	-	+	27	P. coliforme
	AG	-	AG	-	-	AG	AG	AG	AG	AG	-	-	-	-	+	-	-	+	8	P. coliforme
	AG	-	AG	-	-	AG	AG	AG	AG	AG	-	-	-	-	-	-	-	-	1	,,
	AG	-	AG	-	-	Var	Var	Var	Var	AG	-	-	-	-	±	+	-	-	5	,,
A-average 6-8 days	AG	-	AG	AG	-	AG	AG	AG	AG	AG	-	+	-	+	Var	-	-	+	10	P. intermedium
	AG	-	AG	-	-	AG	AG	AG	AG	AG	-	+	-	+	+	-	-	+	1	P. intermedium
	AG	-	AG	-	-	AG	AG	AG	AG	AG	-	+	-	-	-	+	-	+	1	P. intermedium
Ag-average 14-21 days	AG	-	AG	-	-	AG	AG	-	AG	AG	-	-	-	-	Var	-	+	+	22	P. aerogenoides
	AG	-	AG	-	-	AG	AG	-	AG	Var	-	-	-	-	Var	-	+	-	8	P. aerogenoides
	AG	-	AG	Var	-	AG	AG	-	AG	AG	-	+	-	-	Var	-	+	Var	4	P. aerogenoides

Var = variable AG = acid and gas

All reactions 24 hours unless indicated otherwise.



of their classification will be discussed later.

OBSERVATIONS OF THE CULTURAL AND BIOCHEMICAL REACTIONS OF THE P. AEROGENOIDES GROUP

1. Source and Incidence

41 strains were isolated belonging to this species. Table 8 indicates the sources of these strains.

Table 8

Source of Paracolobactrum Aerogenoides Strains

Source	Number of strains
1. Enteric department	
a. Faeces	33
b. Urine	2
2. General Bacteriology	
a. Sputum	1
b. Wound (location not known)	1
c. Sausage	1
d. Fish #	1
3. Veterinary Laboratory	
a. Mink	1
b. Pig	1

This strain was isolated from the stomach contents of a rainbow trout. The fish was one of a large number which had died in a hatchery pond and was submitted to the Provincial Laboratory of Public Health by the Department of Zoology.

2. Cultural and Biochemical Reactions

These strains were morphologically fairly uniform and indistinguishable from usual Enterobacteriaceae such as Salmonellae and E. coli. They were non-haemolytic, for the most part sluggishly motile, and with one exception non-chromogenic. One strain #M2533 produced a lemon yellow pigment, but since in all other respects it

Table 9

Biochemical reactions of P. aerogenoides

Arabinose	Rhamnose	Maltose	d-Tartrate	Citrate	Indole	M.R.	Litmus milk	No strains	Strains included
AG	AG	-	+	-	-	±	NC or A	3	28,162,295
AG	AG	-	+	-	-	±	Alk	1	780
AG	AG	AG	+	-	-	-	NC or A	2	35,113
AG	-	-	+	-	-	-	NC	1	14
AG	AG	-	+	-	-	-	A	2	118,177
AG	AG	-	-	-	-	±	NC	1	161
AG	-	-	-	-	-	+	A	1	167
AG	AG	AG	±	-	-	+	NC or A	8	251,267,268,445 515,779,850,AP113
AG	AG	AG	±	-	-	+	Alk	2	AP111,924
AG	-	-	-	-	-	+	Alk	1	281
AG	A	AG	±	-	+	+	AC	1	2533
AG	AG	AG	±	+	-	-	AC	1	328
AG	-	AG	±	-	-	+	NC or A	2	191,M5308
AG	-	AG	±	-	-	+	Alk	1	345
AG	AG	AG	±	-	-	-	NC	1	3560
-	AG	AG	+	-	-	+	Alk	1	494
AG	AG	AG	-	-	-	+	NC	3	803,M5273,M7427
AG	AG	AG	-	-	-	-	NC	1	924

AG = acid and gas
A = acid
NC = no change
Alk = alkaline
AC = acid and clot

All strains produced acid and gas in glucose, mannite and xylose in 24 hours. Lactose, sucrose, salicin and inosite were not fermented in 24 hours.

conformed with the specific definition, it was tentatively retained in this group.

Strain # 28 serves well as the type culture. It was a gram-negative, non-sporing, motile bacillus 2.5μ long by 0.5μ in diameter. Unevenly stained or "barred" forms were fairly frequently seen. Some variation was seen in colonial morphology. Rough forms appeared frequently. These were preceeded by slightly flattened colonies not usually seen in this family. In addition this phenomenon was common to all strains. Approximately half of the strains studied produced small colony variants, which were indistinguishable biochemically from the usual large forms. In liquid media the production of a scum or pellicle on the surface of the media was not uncommon. This was not necessarily associated with roughness and could readily be removed from broth antigens by filtration.

The production of a rather pungent unpleasant odour was common in this species. This odour was not noted in other Paracolobactrum strains and resembled somewhat that of alpha naphthilamine.

The biochemical reactions were fairly uniform. Glucose and mannite were fermented in 24 hours with the production of acid and gas. Sucrose, dulcite, sorbite and inosite failed to be fermented in the same time. 3 strains gave faint reactions in salicin in 24 hours.

Lactose fermentation was very slow and with two exceptions only minute amounts of gas were produced. Acidity was first evident in the Durham tube and did not appear until at least the

eighth day and in most cases it took from 14 to 24 days to appear. 6 strains had still failed to produce fermentation at the end of 30 days.

Strains #328 and #M2533 were the only ones which clotted milk although a number of the other strains produced acid. At the end of twelve days 11 strains had produced acid in litmus milk and three had rendered the medium alkaline. The rest of the strains produced no observable change in this time.

Table 9 gives the detailed biochemical reactions of this species. Strains #328 and #M2533 varied in several respects from the other strains included.

With the exception of these two strains already mentioned, #328 and #M2533, the former citrate positive and the latter indole positive, these reactions are fairly uniform.

OBSERVATIONS ON THE CULTURAL AND BIOCHEMICAL REACTIONS OF THE P.

COLIFORME GROUP

1. Source and incidence of strains

67 strains were isolated belonging to this species. The sources of these strains were more varied than those of either the P. intermedium or the P. aerogenoides. These sources are listed in Table 10.

Table 10.

Sources of Paracolobactrum Coliforme Strains

Source	No. strains
Faecal	46
Urinary	2
Sputa	3
Vaginal swabs	1
Abdominal abscess	1
Fish #	1
Veterinary Laboratory	13
	67

From the same specimen as the one mentioned in Table 8. Both P. aerogenoides and P. coliforme were isolated from the same specimen.

2. Cultural and Biochemical reactions

Strain # 82 serves well as the type culture within this species. It was a gram negative non-sporing rod of 2 μ in length by 0.5 μ in diameter. It grew well on ordinary solid media giving the usual "coliform" type of colony. It was non-haemolytic on blood agar.

The majority of the strains appeared to be somewhat shorter

than is usual for the Enterobacteriaceae. 3 strains were slightly chromogenic, producing a lemon yellow pigment.

In the classification of the Enterobacteriaceae by Norman et al (1944) the primary division was on the basis of chromogenesis. Chromogens were defined as organisms which were "ordinarily chromogenic at 25°C to 30°C producing pink, rose, red or orange-red pigment, but occasionally non-pigmented." In speaking of the non-chromogenics they stated that "infrequently types or variants show yellow to orange pigment".

According to these statements then, the inclusion of such strains is permissable, if other qualifications are met.

Two strains were haemolytic on blood agar, the haemolysis being of the "beta" type.

All strains fermented glucose and mannite with the production of acid and gas in 24 hours. The fermentation of lactose was delayed. The time varied from two days to sixteen. Three strains had not fermented lactose at the end of the incubation time, which was three weeks and actually only one strain attacked the sugar in 48 hours. Interestingly, this strain and ten others which fermented lactose in three days were found on re-examination about a year later to have developed the capacity to ferment lactose with acid or acid and gas in 24 hours. One third of the strains produced gas as well as acid in lactose and most of the rest produced as small bubble of gas. Sucrose, dulcite, inosite and salicin failed to be fermented within 24 hours. 7 strains were able to ferment salicin slowly. Delayed fermentation of sucrose was not determined.

Table 11

Biochemical Reactions of P. coliforme

Salicin	Arabinose	Xylose	Sorbitol	Rhamnose	Maltose	Inosite	d-Tartrate	Indole	Methyl Red	Litmus milk	No strains
occ Ag	AG	AG	Var	AC	Var	-	+	+	+	AC	15
occ Ag	AG	AG	AG	AG	Var	-	+	+	+	Alk	4
-	AG	AG	Var	AG	Var	-	+	+	+	NC	5
-	AG	-	-	-	AG	-	+	+	+	NC	1
occ Ag	AG	AG	AG	Var	AG	-	+	+	-	AC	2
-	AG	AG	-	Var	AG	-	-	+	+	AC	4
occ Ag	AG	AG	-	AG	AG	-	-	+	+	NC	5
-	AG	-	-	-	AG	-	-	+	+	NC	2
-	-	-	-	-	AG	-	+	+	-	NC	1
occ Ag	Var	AG	-	AG	AG	-	+	-	+	AC	2
-	-	AG	AG	AG	AG	-	+	-	+	Alk	1
-	AG	AG	Var	Var	AG	-	+	-	+	NC	4
-	AG	AG	AG	AG	AG	-	+	-	-	NC	1
-	AG	Var	Var	Var	AG	-		-	-	NC	3
-	AG	-	-	-	AG	-	-	-	+	NC	1
-	-	AG	AG	-	-	-	-	-	+	NC	1

AG = acid and gas
Var = variable
AC = acid and clot
NC = no change
Alk = alkaline
occ AG = occasional acid and gas

All strains fermented glucose and mannite producing acid and gas in 24 hours. Sucrose and Inosite were not fermented in 24 hours. Liquefaction of gelatin and fermentation of lactose were variable.

Table 11 gives more detailed biochemical reactions encountered within this species.

Indole was formed by 75% of strains isolated. Acid and clot was produced in litmus milk by 23 strains and 5 strains produced an alkaline reaction in the same medium. The remaining strains produced no observable change in litmus milk in three weeks. The majority of strains producing acid and clot in litmus milk also produced indole. 11 strains liquefied gelatin.

Table 11 indicates that there was more biochemical variation within this species than within either of the other species of the genus Paracolobactrum. The variation was greatest in the indole negative group. This variation might be thought sufficient to raise some query on the validity of the species as presently defined. This variation will be discussed later.

OBSERVATIONS ON THE CULTURAL AND BIOCHEMICAL REACTIONS OF THE P. INTERMEDIUM GROUP

1. Incidence

16 strains isolated belonged to this species. Of these, 12 remained available for special study. Eleven were isolated from faecal specimens and the other one was received from the Provincial Veterinary Laboratory.

2. Cultural and Biochemical Reactions

The first strain encountered, #1a, served well as the type culture for this species. It was a gram-negative, non-sporing rod 2μ long and 0.5μ in diameter. It grew well on ordinary media producing the usual "coliform" type of colony. It was non-chromogenic and on blood agar non-haemolytic. In common with the other strains studied it produced more than the usual amount of turbidity in liquid media. This was accompanied by the production of some sediment. Strain #1a, in common with most of the other strains studied, produced an unpleasant odour, which could be described as "mildly putrid".

Table 12 indicates the biochemical reactions of this strain and the other strains belonging to the same species. It will be recalled that the particular criteria for inclusion in this species were a negative Voges Proskauer reaction and a positive citrate reaction in 24 hours. Strain #1a, in common with the other members of the species, failed to ferment inosite or salicin, but

Table 12

Biochemical Reactions of P. intermedium

Strain	Lactose	Dulcite	Suc, Sal Inos.	Ar,Xyl,Sor Rham,Malt	d-Tartrate	Methyl Red	Indole	Gelatin	Litmus milk	H_2S.
#89	A7	AG	-	AG	-	+	-	-	AC	+
#95	A7	AG	-	AG	±	+	-	-	AC	+
#183	A8	AG	-	AG	-	+	-	-	AC	+
#213	A7	AG	-	AG	±	+	-	+8	AC	+
#314	Ag6	AG	-	AG	-	-	-	+10	AC	+
#435	A6	AG	-	AG	±	±	-	-	AC	+
#1	A67	AG	-	AG	±	+	-	-	AC	+
#301	A10	-	-	AG	-	+	-	-	AC	+
#831	A6	AG	-	AG	±	+	-	-	AC	+
#123	Ag6	-	-	AG	±	+	+	+8	AC	-
#276	A7	AG	-	AG	-	+	-	+10	AC	+
#AP30	A8	AG	-	AG	-	+	-	-	AC	+

Suc = Sucrose
Sal = Salicin
Inos = Inosite
Arab = Arabinose
Xyl = Xylose
Sorb = Sorbite
Rham = Rhamnose
Malt = Maltose
AG = acid and gas
Ab = acid and bubble of gas
± = doubtful reaction

All strains fermented glucose and mannite producing acid and gas in 24 hours. Maximum length of incubation of lactose, gelatin and litmus milk was 11 days.

did ferment arabinose, xylose, rhamnose and maltose with the production of acid and gas. As indicated in Table 12, fermentation of dulcite varied with the strains.

Lactose was fermented by the eighth day, but rarely was much gas produced by this time. In fact, gas did not appear in any significant amount even after another week's incubation. Sucrose fermentation, if it took place at all, was much slower than lactose. Sucrose tubes were incubated for two weeks and there was only a very faint suggestion of colour change. This did not appear in all tubes and was not sufficient to indicate definite acid production. Hydrogen sulphide was produced in large amounts by all strains except #123 which was also the only strain producing indole. Some strains were able to liquefy gelatin partially, but this ability was not general and was at best slow and weak. All strains were motile.

Litmus milk was acidified and clotted by all organisms. The time required was a little longer than that for the fermentation of lactose.

Strain #123, in spite of its positive citrate reaction and negative Voges Proskauer, in many respects appeared to be more closely related to the P. coliforme group. It acidified and clotted litmus milk more rapidly than any of the other strains. This fact in conjunction with the positive indole reaction and the negative hydrogen sulphide suggest the P. coliforme rather than the P. intermedium species.

3. Biochemical Relationships to the Salmonella genus

A comparison of the biochemical reactions of the P. intermedium species and the genus Salmonella indicates the close resemblance between the two. This is illustrated in Table 13.

Table 13

Biochemical Reactions of Salmonellae and Paracolobactrum Intermedium

	#Salmonellae	P. intermedium
Fermentation of lactose	negative	AG5
Fermentation of glucose, mannite, dulcite and sorbite	AG	AG
Fermentation of sucrose	negative	negative
Utilization of d-tartrate	positive	faint positive
Utilization of citrate	positive	positive
Production of H_2S	usual	positive
Production of indole	negative	negative
Production of acetylmethylcarbinol	negative	negative
Gelatin liquefaction	seldom	slow-partial

as listed in Topley and Wilson

With the exception of strain #123, the reactions within this group are quite uniform. The classification of strain #123 will be discussed later.

OBSERVATIONS OF THE PROTEIN METABOLISM OF THE GENUS PARACOLOBACTRUM

The protein metabolism of a number of species of Paracolobactrum was determined by "paper chromatographic" methods in an investigation by Yanda (1952) carried out concurrently with the one reported here. The strains used were the same as the ones examined by the author. The metabolism of all the P. aerogenoides strains, and of nine each of the P. coliforme and P. intermedium was studied by this method.

Table 14 shows the general pattern of the protein metabolism as determined by this method.

Table 14

Protein Metabolism of Paracolobactrum strains

	B	Asp	Ser	Gl.A.	Alan	Val	Leu
P. aerogenoides	+	-	+	±	±	-	-
P. coliforme	-	occ+	+	±	±	-	-
P. intermedium	-	+	+	±	-	-	-

B = Basic amino acids lysine, arginine, histidine
Asp = Aspartic acid
Ser = Serine
Gl.A= Glutamic acid
Alan= Alanine
Val = Valine
Leu = Leucine
\+ = utilized in metabolism
occ+= occasionally utilized

A personal communication from Miss Yanda, states that she found this pattern to be stable in almost all cases. She further states that the P. aerogenoides species, without exception, produced on tryptophane assay medium (Difco) a polypeptide just below the proline group. This phenomenon was not observed in any other strains examined. The substance produced was not chemically identified.

The two strains #328 and M2533, which were biochemically atypical of the P. aerogenoides group, also produced this material, but in reduced amounts.

In other respects, however, they did not conform to the general pattern of protein metabolism for the group.

A detailed study of the amino acid utilization by the P. aerogenoides group was carried out in the same investigation. Some similarity in the utilization of alanine was detected within the following pairs and groups of strains:

1. #251 and #345
2. #28 and #445
3. #850 and #919
4. #177, #267, #924 and #AP113
5. #191, #113, #921, #M7427 and a 1949 strain.

In the investigation of the antigenic composition of the P. aerogenoides group which follows, some similarity may be seen between these groups and those whose somatic antigenic makeup is related.

PRELIMINARY DISCUSSION OF THE CULTURAL AND BIOCHEMICAL CHARACTERS OF THE GENUS PARACOLOBACTRUM

The morphological and biochemical reactions of the strains studied conformed to the genus Paracolobactrum as defined by Borman et al (1944). The boundaries of this genus, as described by them are wide and the main point of differentiation from the Salmonella genus is the ability to ferment lactose slowly. While this difference between the two genera is not readily discernable, other differences became evident from the morphological and biochemical studies undertaken.

All strains placed in the Paracolobactrum genus failed to ferment inosite. None were able to utilize urea as the sole source of nitrogen and were therefore not members of the Proteus group. The three different groups within the genus presented certain reactions characteristic of each species which, when combined with the general generic reactions, served to differentiate them from other species and genera. While the pattern of protein metabolism did not demonstrate a pattern characteristic of the whole genus, it did demonstrate patterns for each group which were different from those of other genera studied. A consideration of each group separately will indicate more clearly the differences between the two genera.

Strains placed in the P. aerogenoides group showed definite continuity in cultural and biochemical characters. They were characterized by the production of a particular odour, by peculiar colonial variations, and by the ability to produce acetyl-

methylcarbinol in large quantities. They were also able to synthesize a particular protein (polypeptide) material which was again characteristic of the group.

Two strains # 328 and #M2533, whose biochemical reactions and protein metabolism patterns varied somewhat from those of the rest of the group, were able to produce this particular protein, although in reduced quantities. In general, the morphological and biochemical characters of these strains together with the protein metabolism patterns are sufficiently uniform and sufficiently distinctive to warrant their incorporation in the species. The relative importance of certain characters in establishing this species pattern will be classified in a later serological study. The classification of the two "aberrant strains" # 328 and #M2533 which are for the present tentatively left in this group will then be re-considered.

The reactions of the P. coliforme group were not so clear cut. While the protein metabolism was similar in all strains studied, it was investigated in only approximately one quarter of the strains used in my investigation. In biochemical reactions (Table 11), the variations were considerable and occasional reactions (e.g. fermentation of lactose) unstable. The variation in biochemical characters was not distinctive enough to suggest the formation of sub-groups. The difficulty in establishing a uniform relationship in strains ostensibly belonging to the P. coliforme (Borman et al) raises the doubt of the validity of the species. It is felt that the strains included in this group can be classified as members of the Paracolobactrum genus but until serological or other evidence is forth-

coming, they cannot be considered as one species.

With one exception, the P. intermedium group showed definite continuity in cultural and biochemical reactions as well as in the pattern of protein metabolism. As already indicated, a number of investigations of this group have been made by other workers and four sub-groups have been suggested. Their work, in conjunction with the very limited results reported here indicate the validity of this group. According to the classification of Borman et al, the utilization of citrate is the distinguishing character of the group. It is felt however, that, for the time at least, this group should be limited to hydrogen sulphide producing and citrate positive strains. According to the work of the other investigators all strains comprising the four groups just mentioned are producers of hydrogen sulphide. Strain # 123, the one citrate positive, hydrogen sulphide negative strain which was studied in this group, was in most other respects more closely related to the P. coliforme group. Since the validity of the P. coliforme species is questioned here, further work may indicate that before final species division some citrate positive strains should be included in this group.

The results of this biochemical investigation indicate that the use of a "basic" set of fermentation reactions, lactose, glucose, sucrose, and mannite, a medium for the detection of hydrogen sulphide production, another for the determination of citrate utilization, as well as the simple biochemical tests for the production of indole and of acetylmethylcarbinol are sufficient in most cases to differentiate between the species.

P. aerogenoides

M.R. - negative, Citrate - negative
V.P. - positive

Salmonella

M.R.- Positive, Citrate - Positive
V.P. - negative

With the following basic 24 hour biochemical reactions, absence of fermentation of lactose or sucrose, acid and gas in glucose and mannite, no hydrogen sulphide production and a positive Voges Proskauer test (for acetylmethylcarbinol) identifies the P. aerogenoides group. With the same basic reactions, a positive indole test and failure to utilize citrate identifies 75% of the P. coliforme group.

The identification of the P. intermedium group presents a different problem. Their close resemblance to the genus Salmonella on primary isolation has been already pointed out. No definite means of ready differentiation has been determined. Practically two points were noted, however, one was the production of a characteristic odour, the other was the production of an unusual amount of turbidity accompanied by sediment in liquid cultures. These characters appeared, from the very limited investigation carried out here, to be fairly common to the group. These two characters, particularly when accompanied by negative agglutination reactions with polyvalent Salmonella antisera, seemed to indicate P. intermedium strains. Obviously, because of the close resemblance to the Salmonella genus, the "nuisance value" at least of this species is very great.

Since serological studies are often of value in the determination of species, they will be considered in the following section before a final evaluation of Borman's classification is made.

PART FOUR

A SYSTEMATIC STUDY OF THE SEROLOGICAL RELATIONSHIPS OF THE GENUS *PARACOLOBACTRUM*

OBSERVATIONS OF SEROLOGICAL RELATIONSHIPS WITHIN THE P. AEROGENOIDES GROUP

1. Choice of species for special study

The results presented in the foregoing section of this investigation indicate that the species P. aerogenoides possesses some interesting morphological and biochemical characters. It also shows continuity in the biochemical reactions. P.coliforme did not show such continuity, nor did it appear to possess any distinguishing morphological characters. The antigenic pattern of the species P. intermedium has, as indicated on page 6 , already been studied in detail by a number of workers. Accordingly, P. aerogenoides was the species of choice for a detailed serological study.

2. Preliminary investigation

Before proceding with a detailed study of the somatic antigenic pattern, it was deemed advisable to determine the presence of the "K" antigens as described by Kauffmann (1947). These are the so-called "envelope" antigens and the cause of "O" inagglutinability in many instances. An "O" antiserum, prepared against strain #28, gave strongly positive agglutinations with a living suspension of of the same strain. This indicated that this strain at least did not contain these "K" antigens. A further group of eight somatic antisera were prepared and tested in the same fashion. Since there was still no evidence of the presence of "K" antigens, the preparation of "O" antisera was continued and the problem of "K" antigens was, for the time at least, not further considered.

3. Preparation and testing of Somatic antisera

Antisera were prepared against 26 strains of P. aerogenoides. In all cases, antisera of satisfactory titres, varying from 1/400 to 1/6400 were easily prepared. Difficulties were experienced, however, in the preparation of antisera for two other strains #251 and #267. The first rabbit receiving strain #267 died from an intercurrent infection. The second and third animals died shortly after the initial injections in spite of the fact that the primary injection for the third rabbit had been reduced to 0.25 ml. Further attempts to produce an antiserum were not made.

Two rabbits given strain #251 died shortly after the primary injection. In this instance however, the third attempt was successful and a satisfactory antiserum was obtained after the usual course of injections. The fact that an attempt to produce an antiserum from still another strain isolated late in the year 1949 gave the similiar result, indicates that at least three of the strains studied possessed a factor which was markedly toxic to rabbits.

Using tube agglutination methods, each antiserum was tested against 34 strains of P. aerogenoides. These included all strains used for antiserum production and the other strains maintained for special study. The titres were determined on all positive reactions.

Absorption tests were done in all cases in which a positive titre of 1/200 or more was obtained. Antisera were re-examined after absorption to determine the efficiency of the procedure.

4. Results of "O" agglutination tests and relationships detected.

The results of the "O" tube agglutinations are recorded in Table 15. Only four antisera failed to agglutinate any but their homologous antigens. These were strains #295, # 328, # 780 and #M2533. The antiserum of strain #515 failed to agglutinate any heterologous antigen to a titre of more than 1/50. All other antisera gave positive agglutination tests with some of the heterologous antigens to a dilution of at least 1/100.

Table 16 gives the results obtained in the absorption tests.

Three pairs of strains had identical somatic antigens, as indicated by mirror absorption tests. These strains were #28 and #445, #251 and #345, and #281 and #921. Strains #850 and #919 possessed closely related somatic antigenic factors.

In addition relationships existed between the following four groups.

I #113, #117, #191, #267, #268, #281, #779, #921, #924 and #M7427

II #118, #850, #919, #AF113, and #M5308

III #28, #445, and #780

IV #35, #494 and #AF111

Note ; In Table 15 and 16 following, titres are expressed as reciprocals of dilutions.

Antisera \ Antigens	28	445	251	345	281	921	35	494	850	919	113	177	191	268	779	924	M7427	118	AP113	M5308	780	515	162	328	295	M2533	14	267	803	AP111	M3560	M5273
28	6400	6400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-	-
445	3200	6400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-
251	-	-	800	800	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
345	100	-	3200	3200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
281	-	-	-	-	1600	400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3200	-	-	-	-	-	-	-
921	-	-	-	-	200	800	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	400	-	-	-	-
35	-	-	-	-	-	-	3200	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
494	-	-	-	-	-	-	200	1600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-
850	-	-	-	-	-	-	-	-	1600	1600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
919	-	-	-	-	-	-	-	-	3200	6400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1600	-	-
113	-	-	-	-	-	-	-	-	-	-	3200	800	-	400	-	-	-	-	-	-	-	-	-	-	-	-	-	400	-	-	-	-
177	-	-	-	-	-	-	-	-	-	-	-	800	-	100	-	-	-	-	400	-	-	-	-	-	-	-	-	100	-	-	-	-
191	-	-	-	-	-	-	-	-	-	-	-	-	3200	-	400	400	400	-	-	-	-	-	-	-	3200	-	-	3200	-	-	-	-
268	-	-	-	-	-	-	-	-	-	-	-	400	-	1600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
779	-	-	-	-	-	-	-	-	-	-	-	-	800	-	800	-	100	-	-	-	-	-	-	-	-	-	-	800	-	-	-	-
924	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800	50	-	-	-	-	-	-	-	-	-	-	800	-	-	-	-
M7427	-	-	-	-	-	-	-	-	-	-	-	-	-	-	200	200	400	-	-	-	-	-	-	-	-	-	-	400	-	-	-	-
118	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1600	-	-	50	-	-	-	-	-	-	-	-	400	-	-
AP113	-	-	-	-	-	-	-	-	-	-	-	-	100	3200	-	-	-	-	3200	-	-	-	-	-	-	-	-	200	-	-	-	-
M5308	-	-	-	-	-	-	-	-	-	800	-	-	-	-	-	-	-	-	-	1600	-	-	-	-	-	-	-	-	-	-	-	-
780	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	800	-	-	-	-	-	-	-	-	-	-	-
515	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-	3200	-	-	-	-	-	-	-	-	-	-
162	-	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1600	-	-	-	-	-	-	-	-	-
328	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1600	-	-	-	-	-	-	-	-
295	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1600	-	-	-	-	-	-	-
M2533	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1600	-	-	-	-	-	-

Table 16

Antiserum	Antigen	Titre before absorption	Titre after absorption
28	445	6400	--
445	28	3200	--
251	345	800	--
345	251	3200	25 faint
281	921	400	--
921	281	200	--
850	919	1600	--
919	850	3200	200
7427	779	200	--
7427	924	200	--
191	267	3200	--
113	267	800	--
113	177	800	--
AP113	268	3200	--
177	AP113	400	50 faint
268	177	400	--
35	494	200	--
191	295	3200	400
191	779	400	200
924	267	800	100
281	267	400	100
919	AP111	1600	400
M5308	919	800	200 partial
M7427	267	400	100
779	267	1600	100

Table 16 cont'd.

Antiserum	Antigen	Titre before absorption	Titre after absorption
113	268	800	800
118	AP111	800	800
191	924	400	400
191	M7427	400	400
345	295	Antigen	rough
779	191	800	800

During the course of the investigation, strain # 295 became rough. To date all attempts to re-isolate the smooth form have been unsuccessful. Had this antigen remained available and had antiserum from strain #267 been available, these antigenic relationships might have been further clarified.

It is of interest to note that strains #28, #177, #267 from faeces and #268 from urine were all isolated from the one patient at different times, while the strains #779 from faeces and #780 from urine, were both isolated from another patient from specimens collected at the same time. Of the first series #177, #267 and #268, were all from group 1 of the four somatic groups; #28 appeared to have a very different somatic antigenic composition. Of the two strains isolated from the second patient, the faecal strain #779 also fell into the group 1, while the urinary strain #780 showed some relationship to strain #28.

While this may indicate the occurence of two strains in one individual, it is noteworthy that in each case the same

somatic antigenic groups are concerned. Since it is probable that the antigenic composition of these strains is complex, then theoretically at least, it is possible that in the first instance one fraction of the complex antigen is markedly predominant and in the second instance, another fraction predominates.

5. Somatic relationships to strains isolated prior to, and following the investigation.

Using the groups of antigenic factors as listed on page 40 as a guide, three polyvalent antisera were prepared. These included all the antigenic factors found during the investigation. Slide agglutination tests were carried out with the 1949 strain (of faecal origin) and with 12 strains isolated late in 1951 and early in 1952. These latter strains were from a variety of sources and included two from the Provincial Veterinary Laboratory. Table 17 gives the results of these slide agglutination tests.

Table 17

Agglutination tests with Polyvalent "O" antisera and additional strains of E. aerogenoides

Strain	Polyvalent 1	Polyvalent 2	Polyvalent 3
"1949"	++++	--	--
7090/51	--	--	++++
352	--	--	++++
27/52	--	++++	--
44/52	--	++++	--
45/52	--	++++	--
46/52	++	++++	--
161/52	--	--	++++
162/52	--	--	++++
M430/52	--	++++	--
AP1/52	--	--	++++
AP3/52	--	+	+

Tube agglutination tests were done with the individual sera making up the polyvalent antisera where indicated and the titres of positive results determined. Table 18 shows the final results with individual antisera.

Table 18

Agglutinations with individual P. aerogenoides antisera and additional strains

Strain	Antiserum	Titre
1949	177	200
1949	191	1600
1949	281	200
1949	779	800
1949	M7427	400
7080/51	850	800
	M2533	50
	M5308	3200
26/52	AP113	1600
27/52	M35	3200
44/52	162	800
45/52	162	800
46/52	162	800
161/52	5308	25
162/52	AP113	25
	M2533	200
M430/52	162	1600
AP1	M5308	3200
	850	3200
AP3/52	M2533	25
	M7427	50

Two facts can be deduced from the results of the somatic agglutination tests just given.

1. The somatic antigens of P. aerogenoides are complex in nature. This is demonstrated by the fact that in a number of cases where absorption has completely removed an antibody, the "mirror" or reverse reaction has not occured. It is further demonstrated by the fact that in other cases absorption does not completely remove the antibody but merely reduces its titre.

2. Strains isolated before and after the isolation of the investigated strains show, with one exception, antigenic relationship to these strains studied in the investigation.

Table 18 shows that the two strains #7090/51 and #162/52 (both typical P. aerogenoides) show some somatic antigenic relationship to the aberrant strain #M2533. While the antigenic connections are not great, this does demonstrate, in addition to its ability to produce the polypeptide, a further connection of this strain #M2533 to the rest of the P. aerogenoides group. No connections were found between the somatic antigens ofthe strains studied and the antiserum of the aberrant strain #328.

6. Preparation of flagellar antisera

The investigation of the flagellar antigenic relationships was on a much smaller scale than the investigation of the somatic relationships. Only 75% of the 34 strains used previously were motile. Rabbits became unobtainable so that antiserum production was severely curtailed.

Two antisera were prepared, from strains #28 and #494. Tube agglutination tests were done with antigens of all motile organisms and the two antisera. As before, the titre of all positive reactions was determined.

7. Determination of flagellar antigenic relationships

The antiserum of #28 agglutinated only one antigen, that of strain #295. A titre of 1/3200 was obtained in this case and absorption reduced the titre to 1/200.

The antiserum of #494 agglutinated strain #328 to a dilution of 1/400 and that of #M6807 to a dilution of 1/200. In neither

case did absorption reduce the titre. The other "H" antigens were not agglutinated by either of the two available antisera.

The flagellar antigenic investigation is too limited to give any indication of strain relationship. Unless phase variation exists, it does suggest a type variation in "H" antigens within "O" groups, since strains #28 and #445 identical in their somatic antigens, possess different flagellar components.

The fact that the flagellar antigen of the aberrant strain #328 is related to that of #494 is noteworthy. While the antigenic connection is not great, this does demonstrate, in addition to its ability to produce the polypeptide, a further connection of this strain #328 to the rest of the P. aerogenoides group.

The ability to produce the polypeptide and the serological relationships just demonstrated are sufficient to warrant maintaining the two aberrant strains #328 and #M2533 in the P. aerogenoides group.

OBSERVATIONS OF SEROLOGICAL RELATIONSHIPS TO OTHER SPECIES OF PARACOLOBACTRUM

Slide agglutination tests were carried out using the three polyvalent P. aerogenoides somatic antisera prepared earlier and all available P. coliforme and P. intermedium strains. No positive reactions were obtained with the P. intermedium species. Two strains of P. coliforme, #709 and #724 gave positive tests, both with antiserum 111. These strains were tested with the individual members of the polyvalent antiserum and tube agglutinated to a titre of 1/6400 by antiserum #5308 and to a titre of 1/50 by antiserum #2533. Strain #724 was agglutinated to a titre of 1/25 by antiserum #AP113. Antiserum #M5308 was absorbed by strains #709 and this procedure reduced the homologous titre to 1/400. While some relationship exists between the two strains, this shows that the two strains are not identical.

Flagellar agglutination tests carried out using the two antisera from strains #28 and #494 and broth antigens of all P. coliforme and P. intermedium strains were entirely negative.

A flagellar and a somatic antiserum each prepared from strains #314 of the P. intermedium group, failed to give any positive agglutination with appropriate antigens of the P. aerogenoides species.

The flagellar investigation was not of significant magnitude to determine any inter-relationships.

The results of these tests indicate that there is no relationship between the P. aerogenoides strains studied and the strains of P. intermedium that were studied. One instance of a minor

relationship to the P. coliforme group was found. This was not sufficiently significant to be of any importance from a toxonomic viewpoint.

SEROLOGICAL RELATIONSHIPS OF THE SPECIES P. AEROGENOIDES TO THE SALMONELLA AND SHIGELLA GENERA.

1. Investigation of somatic antigenic relationships to the Salmonellae

Slide agglutination tests were carried out using polyvalent Salmonella antisera and all strains of P. aerogenoides. The polyvalent Salmonella antisera included 11 individual Salmonella antisera representing a corresponding number of antigenic combinations. These antisera are sufficient to identify at least 90% of Salmonella strains presently recognized. The exact composition of the antisera is given in the appendix.

Table 19 gives the results of the agglutination tests. Strains not listed in the table gave entirely negative tests.

Table 19

Agglutinations with P. aerogenoides and polyvalent Salmonella O antisera

Strains No.	1	11	Vi
162	++++	--	--
268	++	++	--
295	++++	--	--
494	--	++++	--
780	++	--	--
803	--	+	--
921	++	--	--

Further tests using individual antisera of the pools with which the positive results had been obtained were negative except with strains #494 and #780. The former was positive with anti-

serum 1, XIII, XXIII to a titre of 1/400 and the latter with antiserum XXI, XXVI, to a titre of 1/25.

The original titre of antiserum 1, XIII, XXIII which was 1/800 was not reduced by absorption with strain #494.

The nature of the composition of the polyvalent antisera permits unusually large concentrations of various antigenic factors. When testing is done with individual antisera the proportions are returned to normal. This may offer an explanation for the large number of positive results with the polyvalent antisera in contrast to the small number with individual antisera.

2. Investigation of the flagellar relationships to the Salmonellae

Tube agglutination tests were carried out using polyvalent Salmonella antisera and "H" antigens of the motile strains of P. aerogenoides.

The polyvalent antisera included 16 individual antisera, again sufficient for the identification of 90% of Salmonella strains.

All agglutination tests were negative.

3. Investigation of the relationships to the Shigellae

Tube agglutination tests were done using polyvalent Shigella antisera. These antisera included individual antisera for 19 Shigella strains. They did not include any of the Sachs strains. Their composition is listed in the appendix.

Two positive agglutinations were obtained. Strains #162 was agglutinated by Sh. alkalescens to a dilution of 1/25 and strain AP113 was agglutinated by Sh. sonnei antiserum to a dilution of 1/25.

No major and only very slight minor relationships were found between the species *P. aerogenoides* and the genera *Salmonella* and *Shigella*.

OBSERVATIONS OF THE SEROLOGICAL RELATIONSHIPS WITHIN THE SPECIES P. INTERMEDIUM

A large proportion of the strains of P. intermedium studied had originated in the same institution. The brief histories available suggested that in the majority of cases, these strains were isolated from patients who suffered from intercurrent infections while hospitalized for other illnesses of prolonged duration.

Accordingly antisera were prepared from one of these strains, #314. Tests were then carried out using these antisera and the appropriate antigens. In addition, antigens prepared from one strain since isolated, from the same institution, and from two other serum were included in the tests. Table 20 shows the results that were obtained using "H" and "O" antisera prepared from strain #314.

Table 20

Results of agglutination tests with #314 antisera and strains of similar origin

Antigens	#314 "O" antiserum	#314 "H" antiserum
#89	--	--
#95	--	1/6400 +
#183	--	--
#213	--	1/800
#435	--	--
#34/52	--	--

The results given in Table 20 indicate that the relationship within the group is very slight.

No extensive investigation of the serological relationships within the species was attempted.

Because of the close resemblance biochemically between the Salmonella genus and the P. intermedium species, an attempt was made to determine the serological relationships between the two. All strains were tested against available "H" and "O" polyvalent Salmonella antisera. The composition of these antisera is listed in the Appendix. The antisera included 16 "H" factors and 11 "O" factors, giving a sufficient range of antigenic factors to identify at least 90% of Salmonella strains presently recognized.

When "H" agglutination tests were done, it was found that antigens of #185 and #435 gave slight reactions with z$_{14}$ antiserum. This agglutination might best be described as a partial reaction of the somatic type and for practical purposes it has been disregarded. No agglutinations that could properly be described as flagellar were obtained.

"O" agglutinations were done using the usual slide method. Antigens of strains #123 and #183 had become rough and satisfactory tests could not be carried out. Positive results were obtained with strains #1a and #95, both with polyvalent 1 antiserum. #1a gave a ++++ agglutination and #95 a ++. When these strains were tested against the individual members of the polyvalent antiserum, positive results could not be obtained. As already explained the composition of the polyvalent antisera resulted in an antiserum

with a proportionately high content of factors 1 and XII. The proportionately high concentration of these two factors may explain the positive result in the polyvalent antiserum and the negative result obtained with the individual antisera.

Keeping in mind the fact that only a few strains of this group were studied, these results suggest that although the biochemical relationship to the Salmonella genus is close, the serological relationship is not.

PRELIMINARY DISCUSSION OF THE SEROLOGICAL INVESTIGATIONS

The extensive investigation of the somatic antigenic pattern of the P. aerogenoides species, demonstrated inter-relationships within the group. It also showed that the relationships to the other species and to the Salmonella and Shigella genera were minor indeed. These two observations agree with the cultural and biochemical observations as well as with the observations on protein matabolism in demonstrating the validity of the species Paracolobactrum aerogenoides.

The degree of antigenic variability within the species cannot be assessed from the numbers of strains examined. However, it is evident that there are several comparatively large antigenic sub-groups within the entire species, as well as some small sub-groups. The various unrelated strains encountered may or may not indicate the existence of other small groups.

The biochemical variations within the species did not conform to the somatic grouping. This latter fact is not however significant in other groups. To cite only one example, the antigenic pattern of S. paratyphi C, two varieties of S. cholerae-suis, and S. typhi-suis is almost identical, yet there is considerable variation in their fermentative activities. The study of amino acid utilization as carried out by Yanda gives some strength to the present grouping although time has not yet proven whether we are justified in correlating amino acid utilization and antigenic makeup.

Several facts suggest that the somatic antigens are complex

in nature. Residual antibody often remained in considerable amount after absorption procedures. Recripocal absorption of closely related antisera often gave highly divergent results. The variation in agglutination reactions of strains isolated from the same individual at different times may be significant; it might suggest either a complexity of antigenic makeup with different factors predominating but it could be explained by the presence of two different strains in the same individual. Yet in the case of a rather uncommon organism, this latter possiblity would be at least unexpected.

The results of the investigation failed to demonstrate the presence of any "K" or "envelope" antigens.

It is unfortunate that the flagellar investigation could not be more extensive. Only one fact can be deduced from the results obtained. Unless phase variation exists, strains identical in their somatic antigen are not necessarily completely alike, since strains #28 and #445, identical in their somatic antigenic makeup possess different "H" antigens.

The fact that P. aerogenoides shows only very minor relationships to the P. coliforme group and the Salmonellae and Shigellae emphasizes the validity of the species.

While no serological investigations of the other two groups were carried out, sufficient serological investigation was done to indicate that the I. intermedium species and the indole negative strains presently placed in the P. coliforme group have no major antigenic connections with the genus Salmonella.

PART FIVE

DISCUSSION

DISCUSSION

In this investigation a study was made of the morphological and biochemical reactions and of some of the serological relationships of the genus Paracolobactrum as defined by Borman, Stuart, and Wheeler. An attempt was made to determine the validity of the genus as a whole and of the species within it as suggested by the same authors.

A detailed study of 77 strains and a less detailed study of 42 additional strains apparently belonging to this genus was made. These strains were gram-negative, non-sporing rods which fermented glucose and mannite with the production of acid and gas in 24 hours. They failed to ferment salicin or inosite within the same time. Gelatin was in some instances liquefied but in every instance this reaction was very slow. Urea was not utilized as the sole source of nitrogen in 48 hours. As defined by Borman et al the boundaries of the genus Paracolobactrum are wide and the main point of differentiation from the Salmonella genus is the ability of the Paracolobactrum genus to ferment lactose slowly. While this difference between the two genera is not readily discernible, other differences became evident from the morphological and biochemical studies of the individual groups placed within the genus by the above authors. The three different groups, within the genus presented certain reactions characteristic of each group, which when combined with the general reactions just listed served to differentiate them from each other and from the Salmonella genus.

A report of the pattern of protein metabolism of these three

groups as determined in another investigation carried out concurrently with this one, showed that while there was no pattern characteristic of the whole genus, patterns could be demonstrated for each group which were different from those of other genera studied.

Since serological investigation of the whole genus Paracolobactrum was not attempted, opinions of the validity of the genus can be based only on biochemical and morphological studies, with the assistance of the studies on protein metabolism as carried out by Yanda.

It is felt that sufficient evidence has been given to warrant the acceptance of the validity of the Paracolobactrum genus. Further discussion will indicate that the species as defined by Borman et al cannot all be accepted at present.

The group P. aerogenoides was the subject of the most extensive study in this investigation. From a cultural and biochemical viewpoint, the validity of this species has already been accepted. Two aberrant strains #328 and #M2533 were tentatively placed in the species until serological investigations were completed. The serological studies not only demonstrated much somatic inter-relationship within the group, but also showed only very minor connection to the other groups and to the genus Salmonella. Relationships between the two aberrant strains just mentioned and other members of the group were also demonstrated. These serological relationships confirm the validity of the genus Paracolobactrum aerogenoides and also confirm the tentative identity of strains #328 and #2533 as members of this species.

On page 35 it was stated that strains included in the P. coliforme group could be classified as members of the Paracolobactrum genus. It was also stated that the variation in their biochemical reactions was considerable and not distinctive enough to suggest the formation of sub-groups. Because of the variation in biochemical reactions, the validity of a species Paracolobactrum coliforme was not accepted "until serological or other evidence is forthcoming". Since serological investigation in this regard has not been attempted, these strains must still be considered as Paracolobactrum but the validity of the species P. coliforme must remain unaccepted. The term "coliforme group" is convenient in referring to strains not of the other two species and will be retained for this purpose only.

On a basis of the results of this investigation and those of other authors (page 36) the species P. intermedium was accepted on biochemical grounds, with the reservation that only hydrogen sulphide positive, citrate positive strains be included. Since again, the serological investigation was insufficient, the acceptance of the validity of this species must remain on biochemical grounds alone.

The results of the biochemical section of this investigation indicate the resemblance between the reactions of the genus Salmonella and the genus Paracolobactrum and thus indicate the need for simple and rapid methods of distinguishing between the two genera.

No single test can be accepted as a valid differential point but the use of a "basic" set of biochemical reactions seems to distinguish adequately the great majority of strains and in doubt-

ful cases agglutination tests with a sufficiently wide range of Salmonella antisera will provide confirmatory positive or negative evidence.

The biochemical reactions, in most instances, differentiate, not only between the two genera, but also between the species of Paracolobactrum. Fermentation of glucose and mannite with acid and gas, while lactose and sucrose remain unaffected, and the production of acetymethylcarbinol identifies the species P. aerogenoides. With similiar fermentation reactions, the production of indols, associated with the inability to form acetylmethylcarbinol identifies 75% of the P. coliforme group. P. intermedium strains are not so easily distinguished. While numerically they are not important, their close resemblance to the Salmonellae gives them a "nuisance value" of some magnitude. From the results of this limited investigation, points of practical importance are the production of the typical odour, associated with unusually turbid broth cultures and negative agglutination with polyvalent Salmonellae antisera. All these suggest the identification of P. intermedium. This is usually confirmed by fermentation of lactose in from three to ten days.

Mention of the results of an investigation of the protein metabolism of the genus Paracolobactrum as determined by "paper chromatographic" methods has been included. It helps to confirm the validity of the species P. aerogenoides, and shows some agreement with the somatic antigenic pattern of this species. Results of similar metabolic investigation of the other two species within the genus Paracolobactrum suggested the validity of these two species also but they were based on

studies of only nine strains in each instance. Hence these results are of doubtful value until more comprehensive studies are carried out.

PART SIX

CONCLUSIONS

CONCLUSIONS

1a. The validity of the genus Paracolobactrum as defined by Norman et al was accepted. Although, according to their definition, the limits of the genus are wide, the results of this investigation indicate biochemical and morphological characters of species and groups placed within the genus which serve in most instances to differentiate it readily from the Salmonella genus. The practical value, of the acceptance of the genus of Paracolobactrum is also obvious.

1b. The biochemical and serological results reported here uphold the validity of the species P. aerogenoides. An independent study of the protein metabolism of the genus carried out concurrently with this investigation also helps to confirm the validity of this species.

1c. The very limited results of this investigation uphold the validity of the species P. intermedium provided the definition be enlarged to include the ability to produce hydrogen sulphide.

1d. The biochemical results query the validity of the species P. coliforme as presently defined. It is possible that adequate serological investigation might assist a desicision, but until such information is available the validity of this species cannot be accepted. The study of the protein metabolism just referred to does add some support to the existence of P. intermedium species and also that of P. coliforme, but the extent of the investigation in this respect was too limited to bear much weight.

2. A "basic" set of biochemical reactions serves to differ-

entiate all of the P. aerogenoides and 75% of the strains of the so-called P. coliforme group from the Salmonella genus. It is not sufficient to permit immediate differentiation of the species P. intermedium from the genus Salmonella. The production of a characteristic odour and an unusual amount of turbidity in broth cultures were of some assistance in differentiating between these two latter groups.

3a. Serological investigation carried out indicated much relationship of somatic antigenic factors within the species P. aerogenoides. Evidence was presented to indicate the complexity of these antigens and a possible co-relation between the somatic antigens and the amino acid utilization of certain strains was suggested. Investigation of the flagellar antigenic makeup was limited and sufficiently only to indicate considerable heterogeniety. No evidence was found to indicate the presence of the "K" antigens of Kauffmann. No major antigenic relationships to other members of the genus Paracolobactrum nor to the Salmonella or Shigella genera were demonstrated.

3b. Detailed investigations of the antigenic makeup of the P. intermedium species and the P. coliforme group was not attempted. No evidence of serological relationship was found, however, between the genus Salmonella and either the species P. intermedium or the indole negative strains of the P. coliforme group.

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APPENDIX

I. Composition of Polyvalent Salmonella "O" antisera

1 ml. of each antisera was used and the volume of the mixture was made up to 10 ml. with normal saline. This gave a 1/10 dilution of each of the antisera included.

Polyvalent "O" 1 consisted of;

I, II, XII

IV, V, XII

IX, XII

III, X, XXVI

XXI, XXVI

I, XIII, XXIII, XXXVI

Polyvalent "O" 2 consisted of ;

VI, VII

VI, VIII

(VIII), XX

"Vi" antiserum was used seperately in a dilution of 1/10

II. Composition of polyvalent Salmonella "H" antisera

1 ml of each antiserum was used and the volume made up to 100 ml. with normal saline. This gave a dilution of 1/100 of each antiserum included.

Polyvalent "H" A consisted of;

a, b, d, i, z_{10}, z_{14}

Polyvalent "H" B consisted of;

e,h, g,s,t, m,t,

Polyvalent "H" C consisted of;

k, 1,v, y, z

Polyvalent "H" D consisted of;

1,2,3, 1,5, 1,6,

III. Composition of Polyvalent P. aerogenoides "O" Antisera

1. ml. of each antisera was used and the volume of the mixture was made up to 10 ml. with normal saline. This gave a 1/10 dilution of each individual antiserum used.

Polyvalent 1 consisted of antisera of the following strains;

#28, #113, #191, #281, #345, #779

Polyvalent 2 consisted of antisera of the following strains;

#118, #162, #295, #328, #35, #515.

Polyvalent 3 consisted of antisera of the following strains;

#780, #850, #AP113, #M5308, #M2533, #M 742?

IV. Composition of Shigella polyvalent antisera

Polyvalent antisera were made up so that each individual antiserum was present in a dilution of 1/25. The first 3 antisera listed were used individually.

Antiserum 1 Sh. alkalescens.

" 2. Sh. dispar

" 3. Sh. sonnei

Polyvalent 4. Sh. flexneri 1, 2, and 3.

" 5. Sh. flexneri 4, 5 and 6

" 6. Sh. dysenteriae 1 and 2.

Polyvalent 7. Sh. flexneri X and Y and Sh. boydii 1 and 2

" 8. Sh. boydii 2,3,4, and 6.